Differential distribution of red cell membrane glycoproteins

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DIFFERENTIAL DISTRIBUTION OF RED CELL MEMBRANE GLYCOPROTEINS

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In vitro study of the distribution of membrane glycoproteins during human red cell aging revealed a major glycopeptide band on SDS/polyacrylamide gel incubated at physiological temperature in normal saline or Krebs-Ringer solution without calcium but not with calcium. This glycopeptide appears to result from the transformation of some other membrane glycopeptide.

The molecular organisation of the red cell membrane conforms to a considerable extent with the fluid mosaic model. The membrane glycoproteins of human red cells are rich in sialic acid residues which are responsible for the negative charge at the cell surface.[1] These are involved in M – and N– group specificity.[2] They also have A,I and S group activities.[3] The sialic acid residue of erythrocyte membrane glycoprotein is found to be significantly involved in the process of aging.[4] It has been reported that the storage stress introduces protein changes mainly of a conformational nature affecting shape, hydrophobicities, and protein-protein or protein-lipid interactions.[5] The present study reports an interesting change observed in the distribution of glycoproteins of human red cell membrane during aging '*in vitro*'.

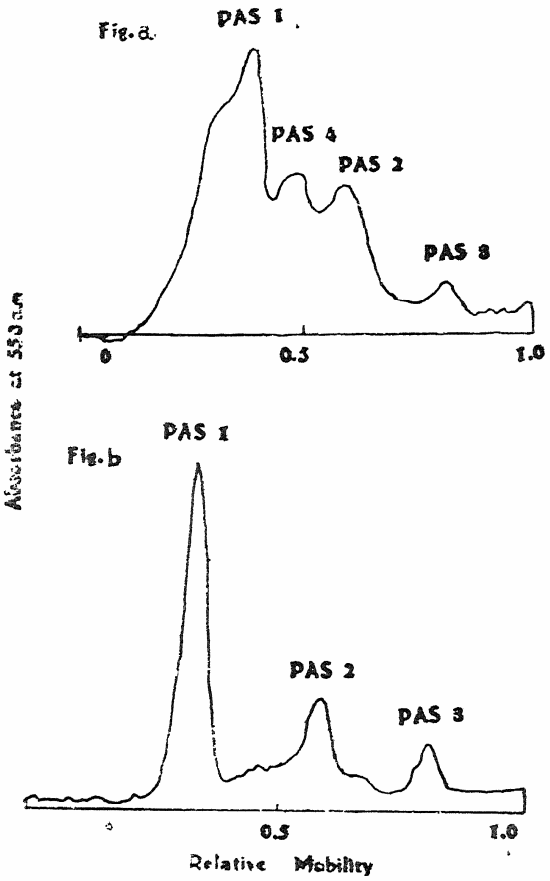
The red cells from human blood were isolated in the same manner as described earlier.[6] The red cells were kept for aging at physiological temperature in different media to see the effects on membrane glycoprotein distribution. Four different media were used : (a) Normal saline (0.154 M NaCl), (b) Normal saline containing 5.55 mM glucose, (c) Krebs-Ringer Phosphate buffer (100 vol. of 0.154 M NaCl, 4 vol. of 0.154 M KCl, 3 vol of 0.11 M $CaCl_2$, I vol. of 0.154 M $MgSO_4$, and 20 vol. of 0.1 M Sodium phosphate buffer, pH 7.4) and (d) Modified Krebs-Ringer solution, containing all ingredients of normal Krebs-Ringer solution except $CaCl_2$.

Four samples (in duplicate), each containing 6-8 ml of red cells suspended in individual media, were taken separately into a dialysis bag (already treated with EDTA and $NaHCO_3$ 1:5, and well-washed with double distilled water) and immersed in about 200 ml of the corresponding medium. These were kept for incubation with shaking at physiological temperature for about 24-30 hours. Control samples were kept at 4°C for the same period. After incubation, each sample was taken out and centrifuged at 25,000g for 15 minutes to get packed red cells. The centrifugation at such a high speed was done to remove membrane free supernatant because a few cells can undergo lysis during incubation with shaking. The settled cells, after above centrifugation, were lysed separately and washed for the isolation of membranes by the method of Marchesi and Palade.[7] Membranes from fresh red cells and those kept at 4°C were also isolated similarly.

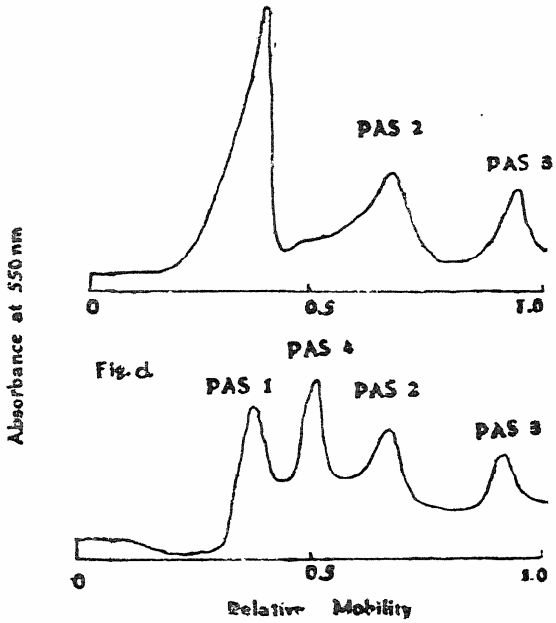
Different membrane preparations were subjected Sodium Dodecyl Sulfate (SDS)/polyacrylamide l electrophoresis. This was carried out on SDS lubilized membranes using the procedure of Fairanks et al.[8] Suspensions of the red cell membrane otein[9] at 0.5-4 mg/ml were prepared for electroioresis by adding the followings (to the stated final oncentration) : 1% SDS, 5-10% Sucrose, 10 mM ris-HCl (pH 8) 1mM EDTA (pH 8), 40 mM DTT Dithiothreitol) and 10 μg/ml of Pyronin Y (tracking dye). These were incubated at 37°C for 15-30 inutes to promote the reduction of disulfide bonds y DTT. Electrophoresis was carried out in an pparatus equipped with convection limiting baffles. bout 250 ml of non-circulating buffer was taken in ach electrode compartment. Each sample (50 μl) was pplied gently beneath the upper buffer onto the top of the gel. The gels of 5.6% cross linking were ised. Electrophoresis was performed at 3.5 mA/tube urrent but initially a current of 0.75mA/tube was sed till the whole sample was completely inside the gel. After electrophoresis the gels were stained for glycopeptides by the periodic acid-schiff (PAS) proedure.[8] The electrophorograms were recorded with . Saitron 803 densitometer at 550 nm and are reproduced in figs, a-d. The glycopeptides have been esignated as PAS 1, PAS 2, PAS 3 and PAS 4.

Four glycopeptide bands were noted in case of amples a and d, three in the case of samples b and c. The relative mobility of each glycopeptide on he gel has been given in Table A. The densitometric pattern of the electrophorogram with the membrane of fresh erythrocytes or those kept at 4°C for 24-30 hours has been shown in fig. b. An interesting observation in this study is the appearance of a PAS 4 component (figs. a and d) representing one of the major glycopeptides in the case of membranes from red cells incubated in modified Krebs-Ringer solution without calcium. Such a band also appeared when the membranes of red cells were incubated in normal saline but it did not appear when the incubation was carried out under similar conditions in Krebs-Ringer solution containing calcium. Evidently calcium inhibits the appearance of the glycopeptide corresponding to PAS 4 in the membrane of red cells. The appearance of PAS 4 glycopeptide in a comparatively high concentration on 24-30 hours incubation of erythrocytes in normal saline of Krebs-Ringer solution without calcium and its disappearance (sometimes appearing as a very small shoulder dim band) when glucose is used in normal saline or Krebs-Ringer solution containing calcium indicates the transformation of other membrane glycopeptides (PAS 1-PAS 2 also called Glycophorin A, or PAS 3 component) into PAS 4 component. Hallam and Wrigglesworth [10] have already reported that Ca^{++} could bind directly to anionic sites on the red cell membrane or alternatively Ca^{++} binding could induce a change in membrane conformation, weakening electrostatic interactions between charged groups on the surface. In red cells Ca^{++} accumulation occurs during physiological aging. It has also been reported[11] that red cells in media containing calcium suffer marked and apparently irreversible aggregation of spectrin, a high molecular weight membrane protein, which has been found to be interconnected with Glycophorin A (PAS 1 and PAS 2), influencing the spatial arrangement of each other in the membrane.[12]

Regarding the incubation of red cells in normal saline containing glucose, it is worth-mentioning that under such conditions there will be an excessive accumulation of 3-phosphoglyceric acid which will influence the metabolic situation of the cell. Scanning of membrane electrophorograms (figs. a-d) prepared from cells incubated in different media also show that after 24-30 hours of incubation, a change in the ratio of bands PAS 1/PAS 2 occurs. Such a rearrangement of membrane components particularly of glycoproteins related to metabolic situation of the cells, therefore, seems to explain to some extent the change in deformability of the red cell membrane.



Figures a,b : SDS/polyacrylamide gel electrophorogroms of human red cell membrane glycoproteins read with densitometer at 550 nm. Fig. a-membrane of red cell incubated in medium a, and Fig.b-membrane of red cell incubated in medium b.



Figures c,d : SDS/ polyacrylamide gel electrophorograms of human red cell membrane glycoproteins read with densitometer at 550 nm. Figc-membrane of. red cell incubated in medium c, and Fig.d-membrane of red cell incubated in medium d.

TABLE A

Relative Mobility* of Erythrocyte Membrane Glycopeptides on SDS/Polyacrylamide Gel.

Experiment No.	Relative Mobility of Individual Glycopeptide			
	PAS 1	PAS 4	PAS 2	PAS 3
I				
Sample a	0.41	0.49	0.59	0.73
" d	0.41	0.50	0.60	0.75
" b	0.42	–	0.61	0.76
" c	0.42	—	0.58	0.76
II				
Sample a	0.41	0.49	0.60	0.75
" d	0.42	0.50	0.60	0.75
" b	0.41	—	0.60	0.75
" c	0.41	—	0.60	0.74
III				
Sample a	0.41	0.50	0.59	0.73
" d	0.41	0.50	0.60	0.73
" b	0.43	—	0.60	0.77
" c	0.43	—	0.60	0.76
Individual Average	0 416	0.497	0.598	0.748
Relative Mobility	±0.0076	±0.0047	±0.0072	±0.0128

*Relative Mobility is calculated as the ratio of the distance travelled by individual glycopeptide and the tracking dye.

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